

TIME CUES FOR SEMILUNAR REPRODUCTION RHYTHMS IN EUROPEAN POPULATIONS OF *CLUNIO MARINUS*. II. THE INFLUENCE OF TIDAL TEMPERATURE CYCLES

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ABSTRACT

Breeding experiments with the intertidal midge *Clunio* demonstrate that tidal temperature cycles combined with the 24-h light-dark cycle can act as time cues controlling the semilunar (syn. lunar-semi-monthly) timing mechanism of the animals, thereby regulating the semilunar emergence rhythm of the population. The two environmental cycles are in identical phase relationships every 15 days. Two types of temperature cycles were examined, sinusoidal fluctuations and short term pulses of 1.5 h (3–5°C amplitude and 12.4 h period each). Comparing the entrained semilunar rhythms in terms of phase relationship to the time cues, the end of the warming interval seems to be the decisive parameter of the tidal temperature changes. The combined exposure to tidal temperature cycles and tidal cycles of mechanical disturbances of the water (an additional time cue in the populations examined) resulted in the correct semilunar synchronization when the temperature rises and the mechanical disturbances alternated as in a natural tidal cycle. The results from three stocks representing different geographical races are discussed in relation to the amplitude of tidal temperature changes in nature, to weak and strong influences of the time cues, to their perception, and to the phase relationship between rhythms and time cues in the experiments and in nature.

INTRODUCTION

The rise and fall of tides cause remarkable fluctuations in the physical environment of intertidal habitats. Among these fluctuations are the change from submergence to atmospheric exposure, the alterations of light intensity and of hydrostatic pressure, the mechanical disturbances correlated with stronger waves and breakers mainly during flooding, and, finally, the fluctuations of temperature. The variations between temperature conditions may be correlated with temperature differences between sea water and exposed substrates (depending on the time of day and the season), or between the higher and deeper layers of the rising and falling waters. However, intertidal organisms have evolved physiological properties that enable them (1) to tolerate these tidal fluctuations of environmental factors within the range of their habitat and (2), at least in many animal species, to time locomotor activity or reproduction by these factors relative to favorable intervals during exposure or inundation (for review see: Neumann, 1976c, 1981).

Populations of the intertidal chironomid *Clunio marinus* Hal. show a special adaptation to their habitat in the lower intertidal range. This adaptation is a semilunar (syn. lunar-semi-monthly) emergence rhythm which enables the immediately reproducing and short-lived midges to synchronize their egg deposition with the time of

spring low water, at a certain time of day about every 15 days when the main parts of the larval habitat are generally exposed (Neumann, 1966). This semilunar emergence rhythm can be induced mechanically in the laboratory by artificial tidal cycles (period 12.4 h) of water disturbances if they are combined with a 24 h light-dark cycle (abbr. LD) such that both cycles have an identical phase relationship, about every 15 days, corresponding to the semilunar period of 14.76 days (Neumann, 1975; 1978). This, however, is valid only for the northern European populations of *Clunio marinus* (above 49°N latitude). The southern populations react to the monthly moonlight cycle which is an unreliable time cue (syn. zeitgeber) in northern latitudes (Neumann, 1966; Neumann and Heimbach, 1979).

Tidal changes in water temperature may also induce a semilunar emergence rhythm as suggested by preliminary experiments (Neumann, unpub.) and by observations of a sheltered *Clunio* population in a location with weak tidal water disturbances (Heimbach, 1976, 1978b). This paper demonstrates the zeitgeber effect of tidal cycles with temperature fluctuations on a semilunar reproduction rhythm.

MATERIAL AND METHODS

The stock "West Norway" used in this study originated from a population near Bergen/western Norway (60°16'N, 5°15'E), and was thoroughly described by Heimbach (1976, 1978b); the stock called "Dorset" came from Studland/southern England (50°39'N, 1°57'W) (Heimbach, 1976, 1978a); and the "Helgoland" stock originated from Helgoland Island in the German Bay/southern North Sea (54°11'N, 7°54'E) (Neumann, 1966, 1978). *Clunio* cultures were bred in air-conditioned rooms (Neumann, 1966). The light-dark cycles were LD 12:12 or LD 16:8. The light intensity for each culture bowl was constant during one experiment and ranged between 500 and 1500 lux, depending on the number of fluorescent tubes and their height above the cultures.

Two different types of artificial cyclic temperature fluctuations (period 12 h, 24 min, 50 s, cor. 12.4 h) were applied, namely "temperature pulses" and "temperature cycles." The experiments with *temperature pulses* were carried out in a tank, containing culture bowls, into which water was pumped from two basins, one dispensing warm water during a 15 min temperature rise, the other dispensing cold water during the rest of the tidal period (Fig. 1a, b). In this experiment great care was taken to avoid any tidal zeitgeber influence by mechanical disturbances (weak vibrations) which might result from the cooling system and the pumps. Control experiments showed that the entrained emergence rhythms were due only to water temperatures. In *temperature cycle* experiments only one basin was used in which the water was warmed over 6 h by a 70 W heating coil. The water was then allowed to cool in the air-conditioned room for the rest of the tidal period (Fig. 1c). A modified temperature cycle with a 15 min heater-induced *temperature rise* of 3–5°C followed by a slow temperature decrease was used in the Helgoland stock experiments (Fig. 8, 10) because some unknown aspect of the experimental conditions associated with the temperature cycles led to high mortality (3 trials).

Experiments testing the zeitgeber influence of artificial cyclic *mechanical water disturbances* were carried out in tanks subjected to vibrations of a synchronous motor (producing underwater sounds of 50–200 cps, 20–30 dB above background noise level). A program of 6 h vibration and 6.4 h silence simulated the tidal pattern of changing turbulence, bottom vibrations, and underwater sound in nature (Neumann, 1978).

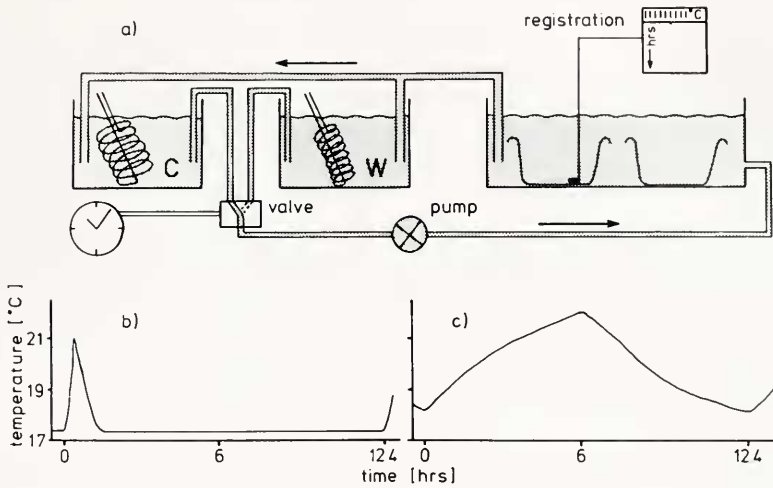


FIGURE 1. (a) Schematic sideview of the apparatus used for triggering a tidal temperature pulse within a tank with culture bowls (right side). C: Basin for cooling the circulating sea water to a standard temperature of about 18°C. W: Basin for warming the circulating sea water to 3–5°C above standard temperature. A valve controlled by a signal clock was switched every 12 h, 25 min to send the warm water into the basin for a period of 15 min. The water was later cooled to the standard temperature circulating through basin C. (b) Shows the resulting temperature cycle.

In the experiments with temperature cycles only one tank was used in which the water was warmed slowly every 12 h, 25 min for a period of 6 h. During the other 6 h, 25 min the water was cooled slowly by the lower room temperature. The resulting temperature cycle is shown in (c).

Temporal control of the artificial tides

The mean period of the semidiurnal tides is about 12.4 h (=12 h, 25 min), shifting daily by about 50 min. The local variations during the synodic month and the seasons depend on the moon's orbit, the influence of the sun's gravitation, and on the geographical characteristics of the sea basin as well as the sea coast. The mean semi-monthly period of the syzygies (full and new moon, correlated at European coasts with spring tides) amounts to 14.76 days, half the synodic month of 29.53 days. Consequently, the spring tides recur about every 14th or 15th day. In spite of these odd periods and local irregularities, it is important to note that the phases of the spring tides (e.g., high water and low water) occur on the average about the same time of day every year (e.g., the mean time of day of spring low waters differs at Helgoland yearly by only a few minutes). To artificially simulate the tides and their semi-monthly recurrence at the same time of day, the semi-monthly period was rounded off to 15.0 days so that registration of emergence days and their evaluation was facilitated. The 29 artificial tidal cycles on these 15.0 days had a period of 12.413 h (=12 h, 24 min, 50 s) in order to reach equal phase relationships with the 24-h LD cycle every 15 days. Thus, each day of the semi-monthly zeitgeber cycle was characterized by a distinct phase relationship of the two zeitgeber factors (LD and tidal factor) (compare Fig. 2). The tidal cycles were triggered by a modified synchronous motor-driven 12-h clock. This clock was combined with two gears (29 and 30 teeth) so that the latter was rotating with a period of 12.413 h as described above (construction by F. Heimbach).

The temperatures in the *Clunio* habitats in Norway (Fig. 9) and Helgoland (Fig. 11) were recorded continuously by thermistors at different habitat locations. The tidal level was read from a marked vertical pole in the water.

RESULTS

When cultures of the *Clunio* stocks were exposed to tidal temperature cycles under strictly controlled laboratory conditions, a clear-cut semilunar emergence rhythm was induced within about 30 days resulting in emergence peaks every 15 days. This is seen in Figure 2 as distinct phase positions of the emergence peaks relative to the semi-monthly zeitgeber program *e.g.*, the tidal temperature cycles and the 24-h LD cycles. In control experiments lacking tidal temperature changes, and using the same environmental chambers, no semilunar emergence peaks were evoked (Fig. 3). Thus, the semilunar synchronization resulted exclusively from the semi-monthly pattern of tidal temperature cycles recurring on each of the 15 zeitgeber days that were in phase with the 24-h LD cycle. However, these experiments do not show which of the 15 zeitgeber days with phase-specific tidal temperature cycles was most effective in the synchronization processes. Nevertheless, the experiments do show that tidal temper-

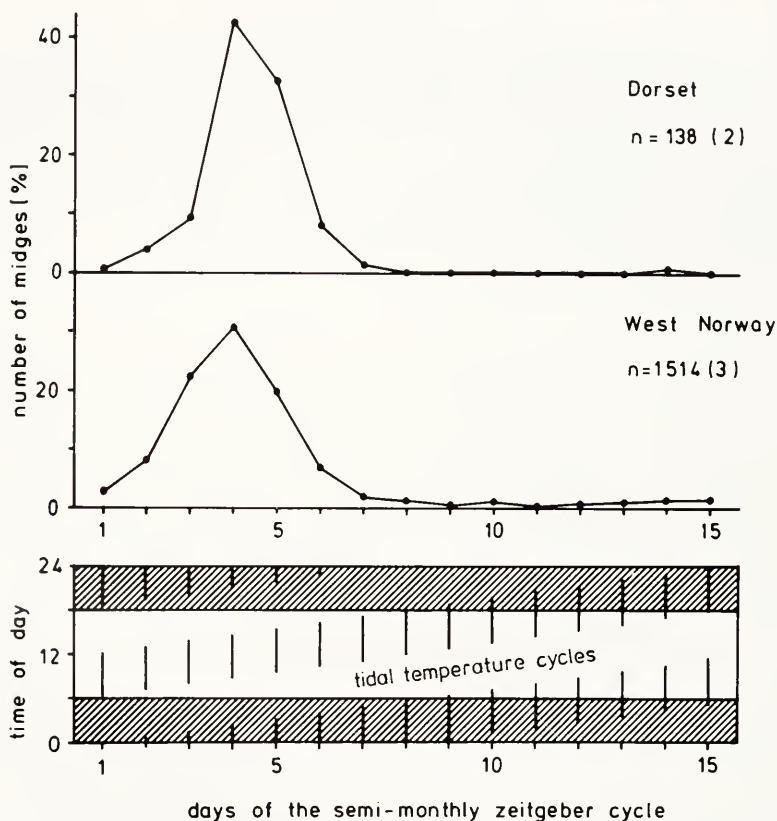


FIGURE 2. Semilunar emergence pattern of the *Clunio marinus* stocks in the LD 12:12, combined with a tidal temperature cycle of 12 h, 25 min duration (as in Fig. 1c) resulting in a semi-monthly zeitgeber program of recurring phase relationships. The upper graphs show the semilunar emergence rhythm of the two stocks, n = the number of midges emerged. The number of successive semi-monthly zeitgeber cycles evaluated (all with the same semilunar emergence pattern) is shown in brackets. The pretreatment by the same zeitgeber program consisted of 2 semi-monthly cycles each.

The graph at the bottom represents the daily warming periods (= vertical lines) during the semi-monthly zeitgeber cycle. Shaded area = darkness.

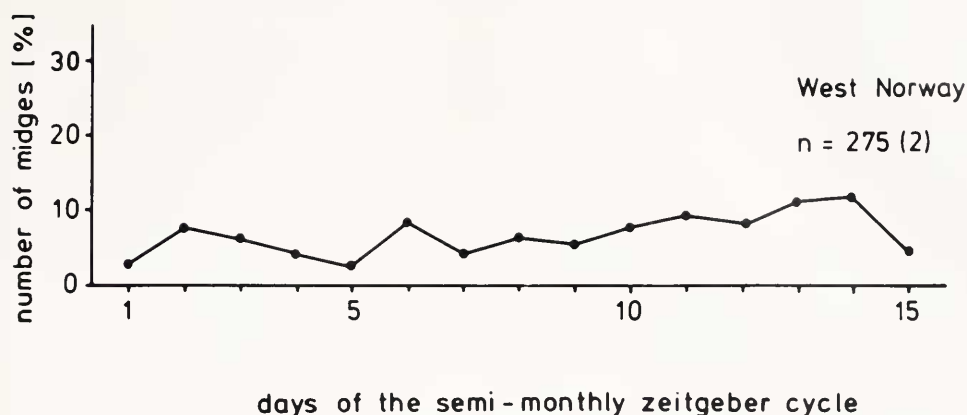


FIGURE 3. Control experiment for the experiments in Figure 2. Conditions: LD 12:12, but the tidal warming period was switched off in the breeding tank so that no tidal temperature fluctuations occurred. The mean temperature was $\sim 18^{\circ}\text{C}$.

ature cycles with an amplitude of a few degrees Celsius may act together with the LD cycle as a zeitgeber for the semilunar-rhythmic emergence days.

Additional experiments evaluated the effect of short tidal temperature pulses (Fig. 1b) designed to simulate short temperature changes that may occur in nature during the time of low water. Even this modified program of tidal temperature fluctuations resulted in a precise semilunar-rhythmic emergence pattern with peaks every 15 days. These days corresponded to the semi-monthly zeitgeber program (Fig. 4).

A true semilunar-rhythmic synchronization of a *Chunio* population by environmental zeitgeber factors depends on an endogenous timing mechanism of the oscillatory "physiological clock" type (Neumann, 1966, 1976a, b). This timing mechanism autonomously oscillates with a period of about two weeks in each larva and permits the pupation in fullgrown larvae only during a few days every 15 days. This temporal control of the developmental processes results in a semilunar rhythm of emergence since the duration of pupation varies only within a small range of 3–5 days at temperatures between 15 and 23°C (Neumann, 1966; Krüger and Neumann, 1983). The effectiveness of this oscillatory timing mechanism is demonstrated when a synchronized population is transferred to conditions of 24-h LD cycles only (without any tidal or lunar zeitgeber conditions) and if a free-running circa-semilunar emergence rhythm of at least two peaks is registered. Such a free-run experiment was undertaken after a synchronization by tidal temperature cycles (Fig. 5). With two peaks at about 15 and 30 days after the last maximum controlled by the zeitgeber program it may be concluded that a circa-semilunar clock mechanism also is involved in these experiments with tidal temperature influences.

The phase relationship between an entrained circa-semilunar *Chunio* rhythm and its entraining semi-monthly zeitgeber program may be additionally influenced by the photoperiod, resulting in a delay of the emergence peak with increasing daylength as previously reported (Neumann and Heimbach, 1979). The same effect of the photoperiod was observed in an experiment with tidal temperature cycles where the delay of the emergence peak was about two days after a change from LD 12:12 conditions to LD 16:8 (compare Figs. 2 and 6, West Norway stock).

Cyclic mechanical disturbances of the water *e.g.*, turbulence, are additional factors which may act as zeitgeber on the entrainment of the semilunar emergence rhythm

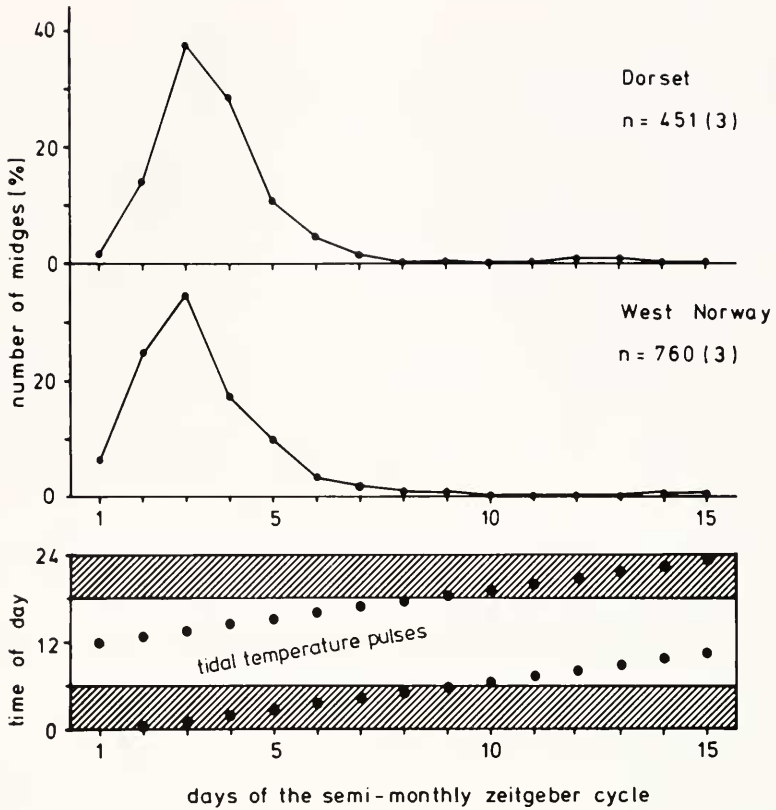


FIGURE 4. Semilunar emergence pattern of the two *Clunio* stocks in the LD 12:12, combined with temperature pulses of about 1.5 h length in regular intervals of 12 h, 25 min (cf. Fig. 1b). The dots in the bottom graph represent the time of day of the temperature pulses. See Figure 2 for further explanation. Data for the Dorset stock from Heimbach (1978a).

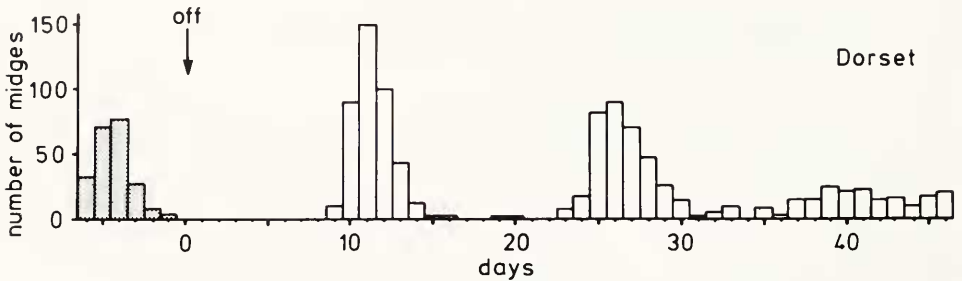


FIGURE 5. Free-running semilunar emergence rhythm of the *Clunio* stock from southern England in the LD 12:12, after entrainment of a semilunar emergence rhythm by the combination of LD 12:12 and tidal temperature pulses for several months as described in Figure 3. Dark columns: the last days of the experiment with temperature pulses; at day 0 the heating was switched off, while the other instruments (valve, pump, cooling, temperature control) remained switched on continuously.

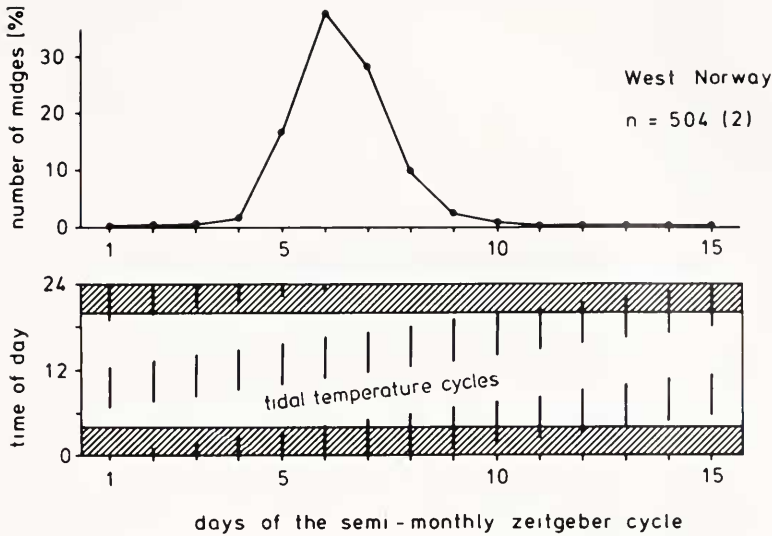


FIGURE 6. The zeitgeber effects of tidal temperature cycles (cf. Fig. 1c) combined with a LD 16:8, resulting in a semilunar emergence pattern in the West Norway stock of *Clunio marinus* (compare with Fig. 2). The temperature level in this experiment was about 3°C higher than in the experiments of Figure 2.

(Neumann, 1978). Three different aspects were considered in the following experiments: (a) strong or weak zeitgeber influence of this (turbulence) factor, (b) the phase relationship of the peaks within the semi-monthly zeitgeber cycle, and (c) the combined effectiveness of both time cues, the tidal cycles of temperature, and mechanical disturbances.

A relatively strong zeitgeber influence of the latter factor was established in the Dorset (Fig. 7, above; standard deviation S.D. = 1.9 days) and Helgoland (Fig. 8, above) stocks. The West Norway stock differed from such a precise synchronization, in that the mean semilunar-rhythmic distribution of the emerged midges was broader (Fig. 7, below; S.D. = 2.6 days). The standard deviation was even enlarged in an experiment with the same tidal program but with LD 12:12 instead of LD 16:8 conditions (no fig.; maximum value with only 14% midges on day 7, and values of about 10% on days 5–6 and 8–10, and relatively high values of 3–6% on the remaining days of the semi-monthly cycle; S.D. = 3.3 days). Mechanical disturbance of the water is apparently a weaker zeitgeber in the West Norway stock, whereas the temperature cycles have a stronger synchronizing effect (Figs. 1, 4). Conversely, the Helgoland and Dorset stocks respond to both zeitgeber factors with a clear-cut synchronization in longer and shorter photoperiods.

Such population-specific differences in the response to artificial zeitgeber conditions have been reported previously in *Clunio* stocks from north and south European coasts with respect to the influence of the monthly moonlight cycle and tidal water disturbances (Neumann, 1966, 1976a, 1978). These differences can be interpreted as the populations' genetic adaptations to reliable zeitgeber conditions of their geographical areas. The West Norway population represents a new type of physiological *Clunio* race that has a specific sensitivity to tidal temperature cycles. This adaptation of its circa-semilunar timing mechanism has ecological significance because clear-cut tem-

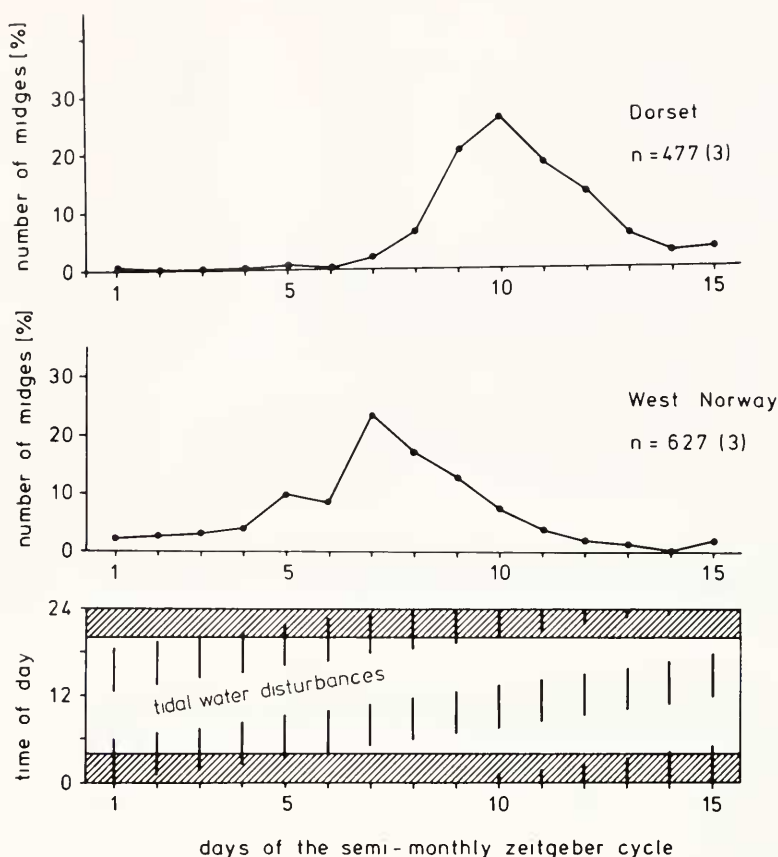


FIGURE 7. The influence of tidal mechanical water disturbances in a LD 16:8 on the emergence pattern of the two *Chnio marinus* stocks from southern England and western Norway. The vertical lines in the scheme below represent the time of daily water disturbances (6 hours every 12 h, 25 min) during the semi-monthly program.

perature fluctuations exist parallel with the tides (Fig. 9), while the changes of mechanical disturbances between ebb and flood are probably weak or often absent in the sheltered fjords with low amplitude tides.

If one compares the synchronization by the temperature zeitgeber with that by mechanical stimulation (Figs. 2, 7, Dorset stock; Fig. 8, Helgoland stock) one discovers differences of several days in the phase of the peaks relative to the semi-monthly zeitgeber cycle, even considering the Dorset experiment where a delay of two days was caused by the photoperiod influence described above. One may ask if these phase differences are merely a consequence of a randomly chosen correlation between both zeitgeber programs, or if they additionally demonstrate different properties of the physiological mechanisms for the perception of the temperature and mechanical zeitgebers. In fact, how both zeitgeber cycles can be exactly related to one another is a problem since in nature both factors reach their maximum values during different tide phases. The rise of temperature occurs during low tide and stops with the following

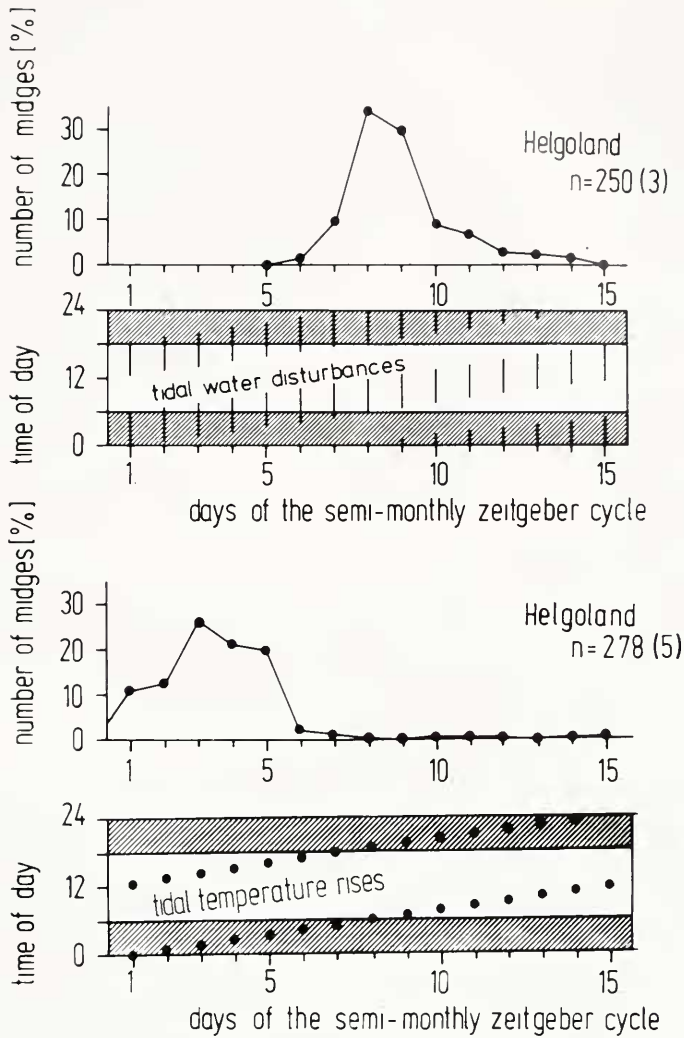


FIGURE 8. Helgoland stock of *Chnio marinus*. Mean semilunar emergence pattern entrained by tidal water disturbances (above) resp. tidal temperature rises (below) in LD 12:12. For comparison of both semi-monthly zeitgeber programs the data have been plotted in such a way that on each day the onset of the water disturbances (above) correlates with the end of the 15 min temperature rises (below).

inundation. The mechanical water disturbances are strongest during the rising flood. The comparison of the simplified laboratory zeitgeber programs with that occurring in nature as well as the correspondence of the phase relationship of the emergence peaks will be examined in more detail in the Discussion. However, in nature both tidal zeitgeber factors act during successive phases of the tidal cycle. Therefore the possibility of synchronizing the semilunar emergence rhythm with both tidal zeitgeber cycles simultaneously was tested in a final set of experiments (Fig. 10).

When mechanical disturbances and temperature increases alternated with each

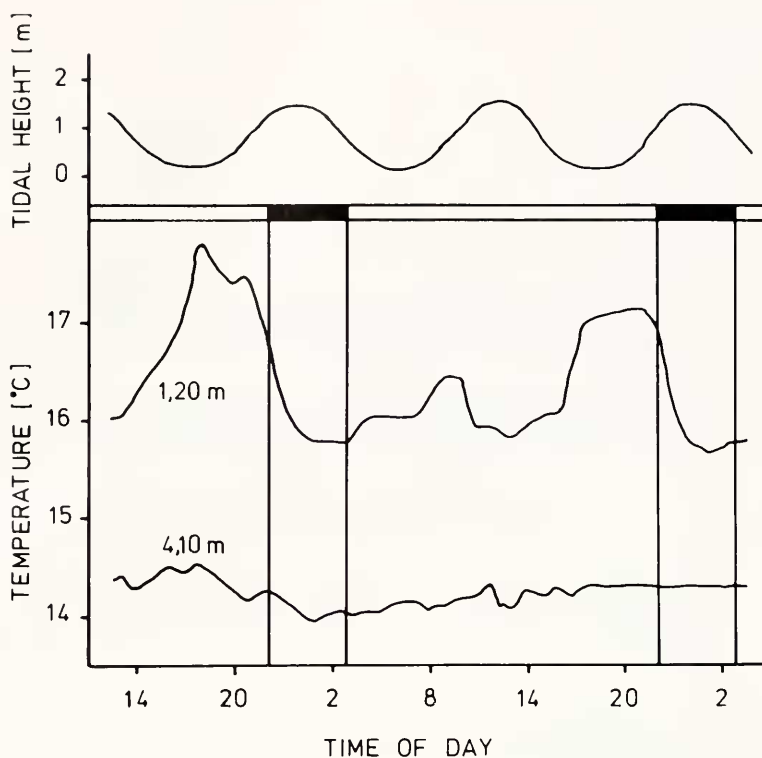


FIGURE 9. Temperature fluctuations in two habitats of different depths in Kviturdvikkollen near Bergen, western Norway in relation to tides and darkness (= black horizontal bar) on 10-8- and 11-8-76 (days of spring tide). The upper temperature curve was recorded at a depth of 1.2 m mean level (corresponding with the boundary between sub- and eulittoral), the lower one at a depth of 4.1 m. There had been sunny calm weather for several days. The maximum air temperatures at Bergen Airport were 21° and 18°C with wind forces 0–3.3 m/s.

other as in experiment 1 (Fig. 10), a normal semilunar emergence rhythm resulted with a peak of about 30% emerged midges at day 8 or 9 of the semi-monthly cycle, as was usual in this standard program with 6 h turbulence per tidal cycle (start of turbulence at midnight on day 1, 18–20°C LD 12:12). A specific reinforcement of the synchronization by the tidal temperature cycles could not be detected (compare Figs. 8 and 10). An irregular distribution of emerging midges resulted in experiment 4 (Fig. 10) when the tidal temperature increases always coincided with the start of the 6-h turbulence signal. In view of natural conditions in the intertidal zone, this was an unrealistic program because in nature the beginning of an inundation with increasing mechanical disturbances by breakers and a temperature rise at low water time differ by several hours. The experiment clearly demonstrates that in such a phase relationship of both tidal factors a mutual repression of their synchronizing effects must have occurred. The two other experiments with intermediate phase relationships of both factors (no. 2 and 3 in Fig. 10) resulted in a deviating entrainment of the semilunar emergence rhythm, with a small tendency for a delayed peak in number 2, and an even weaker synchronization in number 3.

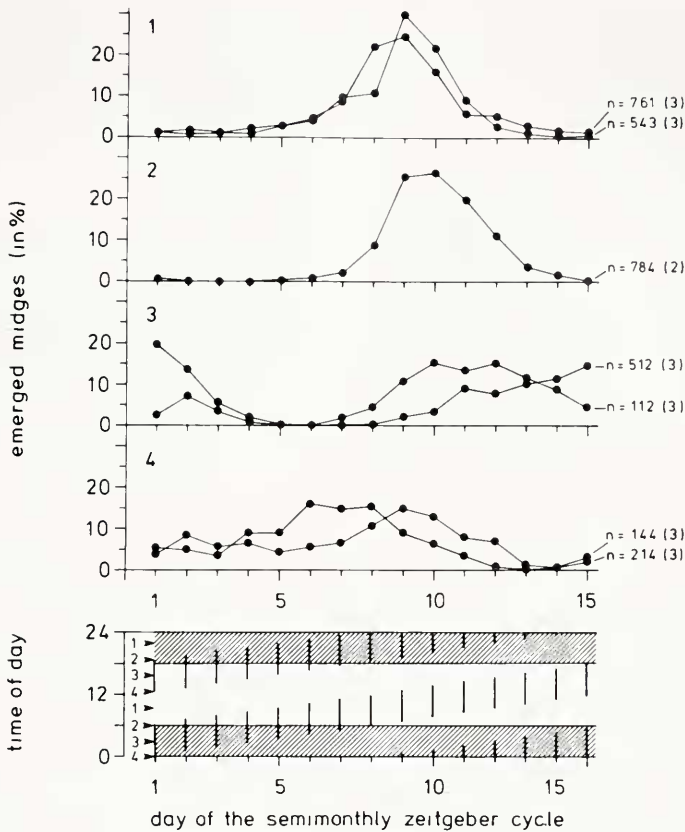


FIGURE 10. Helgoland stock of *Clunio marinus*. Influence of two combined tidal zeitgeber cycles on the semilunar emergence pattern. Both zeitgeber cycles—the tidal water disturbances and the tidal temperature rises—had identical phase relationships with the LD 12:12 every 15 days, but different phase relationships with each other, as shown in the lower graph. Experiment 1: temperature rise between successive tidal water disturbances (middle of simulated ebb); experiment 2: temperature rise at the end of each interval of water disturbances (start of simulated ebb); experiment 3: temperature rise during the middle of the water disturbance interval (midst of simulated flood); experiment 4: temperature rise at the onset of the water disturbance interval (start of simulated flood). The 4 upper graphs represent the mean distribution for emerged midges during the experimental program (n = number of emerged midges; numbers of semi-monthly cycles in brackets). In experiments 1, 3, and 4 two independent series were conducted. Before evaluation of each experiment, the cultures were treated with the same zeitgeber program for at least 30 days. The lower graph represents the tidal water disturbances and standardized phase relationships with the LD conditions.

DISCUSSION

The foregoing experiments demonstrate that temperature fluctuations of 12.4-h period and of 3–5°C amplitude, in combination with a 24-h light-dark cycle, can act as an environmental time cue for the semilunar emergence rhythms of northern populations of the European *Clunio marinus*. These two environmental cycles (temperature and light) result in semi-monthly cycles of recurring phase relationships. Since neither of these periods (12.4 h and 24 h) contains the information for the 15

day period, one must conclude that only a distinct phase relationship of both during the semi-monthly program is effective for the entrainment of the biological rhythm. In the case of the zeitgeber influence of tidal cyclic water disturbances combined with daily light-dark cycles, this conclusion was strengthened by further experiments with modified zeitgeber conditions (Neumann, 1968, 1976b, 1978).

The amplitude of the tidal temperature changes

The influence of temperature change amplitude has not been examined in detail. However, the experimental changes (3–5°C) were within the range of natural conditions, as shown by field measurements. In the larval habitat area of the West Norway population of *C. marinus* (situated between the lower midlittoral and sublittoral of sheltered bays), a change in water temperature of up to 2°C was found parallel with the tidal rise and fall during a period of warm, calm weather (Fig. 9, upper curve). During the exposure of the habitat to air temperatures, the differences were probably even larger, due to higher air temperatures as well as to the solar radiation during daytime. At the *Clunio* location of Helgoland in April and May (spring swarming period), differences of up to 12°C have been registered between the exposed substrates at the time of low water and the mean water temperature (5–8°C). The changes were about 5°C on sunny days when mean water temperatures were 15–18°C (summer swarming period) (Krüger and Neumann, 1983). Similar temperature fluctuations may occur at the Dorset location (Heimbach, 1976). Temperature rises on the order of 2–12°C also have been measured in the upper sediment layers of a tidal mud flat (de Wilde and Berghuis, 1978).

The amplitude of tidal temperature changes will be modified by daily, lunar-semi-monthly, and seasonal variations. The daily variation occurs when the temperature increases mainly from direct solar radiation and fails to appear during night low water. This is valid for the Helgoland location (Fig. 11; compare also Krüger and Neumann, 1983). Lunar-semi-monthly variations of the mean water temperature (period 14.76 days) were found in a small sea area influenced by periodically drying mud flats where the semidiurnal lunar tide interacts with the daily radiation (Mok Bay at the Wadden Sea/Netherlands; Vugts and Zimmermann, 1975). The seasonal temperature variation and daily amplitudes follow seasonal weather conditions. However, the seasonal, as well as the lunar-semi-monthly modulations of the tidal tem-

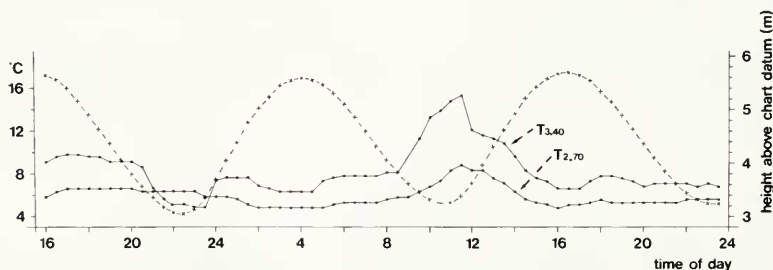


FIGURE 11. Daily variation of the tidal temperature fluctuation at Helgoland on 18 and 19 April, 1983. T-3.40: a thermistor which was located between *Clunio* substrates at 3.40 m height above chart level; it was exposed during low water time. T-2.70: a thermistor, in a water channel nearby, placed below the low water mark at 2.70 m. Air temperatures: 18 April: 3.3°C at 21:30 h, 19 April: 5.0°C at 14:30 h, 4.6 at 21:30 h, wind: SW-S, wind speed: 3.4–5.4 m/s, cloudiness: $\frac{1}{10}$ of sky at 14:30 h.

perature cycles, may be of little or no physiological significance for the entrainment of the semilunar rhythms of *Clunio* because minimum tidal changes of a few degrees Celsius occur on nearly all days during the swarming period (spring until autumn), and because lunar-semi-monthly temperature variations were not required for synchronization in the experiments. Finally, it is difficult to imagine how the insects could detect a lunar-semi-monthly fluctuation in mean daily temperature within the daily temperature fluctuations. Although no such experiments have been performed we suggest that the entrainment of the semilunar rhythm could be reliably triggered if only one of the two tidal temperature rises occurs per day.

Finally, the lower critical amplitude needs consideration. In an arctic *Clunio* population a tidal emergence rhythm was evoked by small temperature changes of only 1°C every 12.4 h (Pflüger, 1973). The circadian locomotory rhythm of the lizard *Lacerta sicula* was entrained in some specimens by a cyclic temperature difference of only 0.9°C (Hoffmann, 1968). Thus, one may expect that even tidal temperature increases of less than 3°C might be effective in the semilunar synchronization of the northern *Clunio* populations.

Perception of the tidal temperature rises

If only a distinct phase relationship between the tidal temperature rise and the day-night cycle is necessary for a semi-monthly zeitgeber program, then one of the physiological components of the *Clunio* timing mechanism for the semilunar control of pupation might be a circadian sensitive perception of the tidal temperature changes within a specific interval of the day. This hypothesis is similar to the zeitgeber perception model of the tidal cycles of mechanical water disturbances during the semi-monthly cycle (Neumann, 1976b). However, the physiological mechanism for perception of temperature changes must involve components other than those described in this model for two reasons: (1) the stimuli of mechanical disturbances were only effective when they lasted for at least 6 h within the 12.4 h period, in contrast to the effectiveness of the short tidal temperature rises; (2) from an ecological point of view, the sensitive circadian phase postulated should be different for both temperature and mechanical disturbance, because temperature increase during low water does not coincide with the effective change of the water disturbance from higher to lower values at the time of high water. This view agrees with the results of the experiment with combined tidal cycles of mechanical disturbance and temperature changes (p. 519).

The shape of the tidal temperature changes

The simulated tidal temperature fluctuations differed in its temporal shape: 15 min pulses and saw tooth curve (Fig. 1, p. 511). They can be characterized by different parameters: by the beginning and ending of the warming period, and by the steepness of the temperature increase and decrease. It is of both ecological and physiological interest to ask which of the parameters correlates with similar or even identical phase relationships of the entrained semilunar rhythms in the experiments. From the results in Table I the decisive parameter of the temperature zeitgeber was the end of the warming period (identical to the beginning of the temperature decrease). In nature this conclusion means that tidal temperature changes might occur at the end of low water when the exposed habitat in the lower midlittoral is again inundated. It is noteworthy that the steepness of the applied temperature increases was of no importance. These deductions should be relevant for any model of the tidal temperature perception within the physiological timing mechanisms of *Clunio*.

TABLE I

The phase relationship between the semilunar emergence rhythm of Clunio marinus and the semi-monthly zeitgeber program in the experiments of Figures 2 and 4 as characterized by the time of day of the tidal warming on the days of maximum emergence

<i>Clunio</i> stock	Tidal temp. cycles in Figure 2		Tidal temp. pulses in Figure 4	
	Start of warming	End of warming	Start of warming	End of warming
Dorset	20.15*	<u>2.15</u>	0.45	<u>1.00</u>
	8.45	<u>14.45</u>	13.00	<u>13.15</u>
West Norway	20.15*	<u>2.15</u>	0.45	<u>1.00</u>
	8.45	<u>14.45</u>	13.00	<u>13.15</u>

LD 12:12, 20°C.

The times of day are rounded off to quarters of an hour.

* Time of day on the evening before.

Comparison of experimental and natural phase relationships

To evaluate the quality of the entrainment of the semilunar rhythms of *Clunio* in the experiments, one should compare the artificial tidal conditions with the natural tides. One can confidently state that the temporal program of the artificial tides (either with temperature changes or mechanical disturbances) did simulate nature well because the peaks of the entrained rhythms recurred within each experiment on the same days as the arbitrarily defined semi-monthly zeitgeber cycle. Thus, it was possible, in each case, to plot the mean distribution of the emergence days as seen in the figures. However, with respect to the phase relationship between the rhythm and the tidal cycles, one must compare the natural and artificial conditions on a specific day, *e.g.*, the day of maximum emergence.

Detailed field observations on the emergence rhythm exist for the locations at Helgoland/North Sea during the summer swarming period. The peak emergence generally occurs on days of spring tides (one day after full and new moons; Caspers, 1951; Neumann, 1966; Neumann and Heimbach, 1979; Krüger and Neumann, 1983). At West Norway the semilunar-rhythmic maxima occurred under equivalent conditions, *i.e.*, days of spring tides about one day after the syzygies (Heimbach, 1976, 1978b). Comparable summer data for the Dorset/English Channel location are lacking. The only Dorset field data from the spring swarming period showed no obvious semilunar synchronization (Heimbach, 1976, 1978a). During April and May the semilunar emergence pattern of this location may be disturbed similar to that of the Helgoland location during spring when the habitat's temperature strongly fluctuates between cold water inundation and exposure to sunny warm weather (Krüger and Neumann, 1983). Thus, only the Helgoland and the West Norway data are given in Table II.

In relation to the chosen phase reference point, the onsets of the artificial tides of mechanical water disturbances differ from the natural tides and their low water time in the range of less than one hour (exactly with a $\Delta t_{B,C}$ of -30 min, Table II). This gives an excellent correlation between both the tidal pattern and the phase of the semilunar rhythm. This holds true even in experiments with a summer photoperiod of LD 16:8 where the emergence peak was delayed by about two days in the Helgoland

TABLE II

Tidal conditions characterized by the time of day of distinct reference points on days of maximum emergency

Tidal conditions	A ¹ Temp. pulses or rises	B ² Mech. disturbance	C ³ Natural tides	
Tidal reference point	End of warming	Onset of mech. disturb.	Time of low water	$\Delta t_{A,C} \Delta t_{B,C}$
Helgoland	Figure 8	Figure 8	Tide tables	
	2.00	5.45	6.15	
	14.30	18.15	18.45	$\sim 4 \text{ h} < 1 \text{ h}$
West Norway	Figure 4	Without Figure		
	1.00	5.00*	4.30	
	13.15	17.30*	17.00	$\sim 4 \text{ h} < 1 \text{ h}$

¹ LD 12:12, $\sim 20^\circ\text{C}$.

² Six hours duration, LD 12:12, $\sim 20^\circ\text{C}$.

³ Local time

* Weak entrainment of the semilunar rhythm.

Times of day rounded off to quarters of an hour, Δt values to whole hours.

stock and the $\Delta t_{B,C}$ resulted in approximately +1 h (Neumann and Heimbach, 1979). Although the entrainment was relatively weak in the West Norway stock, we suggest p. 516), we suggest that the tidal disturbances of the experiments act similarly to those in nature, as a stronger time cue for the Helgoland population, and as a weaker one for the West Norway population.

With regard to the tidal temperature pulses and the end of its warming period, there was a time difference of some hours compared to the natural tides ($\Delta t_{A,C}$ about 4 h in LD 12:12, Table II). In the summer photoperiod of LD 16:8 the emergence peak of the West Norway stock (Fig. 6) shifted to day 6 of the semimonthly cycle where the end of the warming interval was within the next range of the natural low water time (end of warming about 16.45 h, time of MLWS about 17.00 h). Thus, the experimental temperature cycles and the natural tides correlate well. However, in the LD 12:12 conditions there remains a discrepancy of several hours ($\Delta t_{A,C}$ in Table II) which was unexpected because the warming of the exposed habitat should end at about low water with the recurring flood. The abnormal shape of the temperature cycle (15 min temperature rise and slow cooling, p. 510) in the Helgoland experiments (Fig. 8, 10) may complicate a direct comparison of the 'end of heating' parameter with the low water time of natural tides. This tentative interpretation is consistent with the result of experiment 4 in Fig. 10 where the simultaneous zeitgeber effect of such a temperature rise and the onset of water disturbances was abolished. However, all of the experiments demonstrate that artificial tidal temperature cycles with an amplitude of only a few degrees can entrain a semilunar rhythm comparable to that seen in nature. Thus, we may conclude that the European *Chunio* populations have adapted to three different environmental time cues for the semilunar programming: monthly moonlight cycle, mechanical tidal water disturbances, and, as established in the present experiments, tidal temperature cycles. Each of these factors acts together with 24-h light-dark cycle.

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